

WHAT IS CLAIMED IS:

1. A method of generating a thermal property curve for at least one molecule in a microfluidic device, the method comprising:

- 5 (a) flowing the at least one molecule into at least one microchannel or microchamber;
(b) heating the at least one molecule in the at least one microchannel or microchamber;
(c) detecting at least one detectable property of the at least one molecule during the heating; and,
(d) generating a thermal property curve for the at least one molecule.

10 2. The method of claim 1, wherein the at least one molecule changes at least one of its physical properties by unfolding or denaturing, or by altering one or more additional physical property in response to temperature.

15 3. The method of claim 1, wherein the at least one molecule comprises one or more of: a protein, an enzyme, a nucleic acid, a peptide nucleic acid, a ligand, a cofactor, a receptor, a substrate, a single-stranded oligonucleotide, a double-stranded oligonucleotide, an antibody, an antigen, or a polypeptide.

4. The method of claim 3, wherein the at least one molecule comprises a complex of two or more molecules; and wherein the complex comprises at least a first enzyme and at least one of: a second enzyme, a ligand, a peptide nucleic acid, a cofactor, a receptor, or a substrate.

20 5. The method of claim 1, wherein flowing comprises electrokinetically transporting the at least one molecule through the at least one microchannel or microchamber.

25 6. The method of claim 1, wherein flowing comprises transporting the at least one molecule through the at least one microchannel or microchamber under positive or negative pressure.

7. The method of claim 1, wherein flowing the at least one molecule into the at least one microchannel or microchamber comprises transporting at least a first

molecule through the at least one microchannel or microchamber and contacting the at least first molecule in the at least one microchannel or microchamber with at least a second molecule, the method further comprising heating the molecules and detecting at least one physical property of the first molecule, the second molecule, or a complex of the first and the second molecules together while the molecules are heated in the microchannel or microchamber.

8. The method of claim 7, wherein flowing comprises simultaneously transporting the at least first molecule and the at least second molecule through the at least one microchannel or microchamber.

10 9. The method of claim 7, wherein flowing comprises sequentially transporting the at least first molecule and the at least second molecule through the at least one microchannel or microchamber.

10. The method of claim 7, wherein the at least one microchannel or microchamber comprises at least a first microchannel and at least a second microchannel.

15 11. The method of claim 10, wherein the at least first molecule is transported through the at least first microchannel and the at least second molecule is transported through the at least second microchannel.

12. The method of claim 11, wherein the at least first microchannel intersects with the at least second microchannel.

20 13. The method of claim 1, wherein heating comprises elevating the temperature of the at least one molecule for a selected period of time.

14. The method of claim 13, wherein the selected period of time comprises about 0.1 second to about 1.0 second or more.

25 15. The method of claim 13, wherein the selected period of time comprises about 0.1 second to about 10 seconds or more.

16. The method of claim 13, wherein the selected period of time comprises about 0.1 second to about 1.0 minute or more.

17. The method of claim 1, wherein heating comprises commencing heating the at least one molecule at a selected point in time after contacting the at least one molecule by at least a second molecule.

18. The method of claim 17, wherein the selected point in time comprises about 0.1 second to about 1.0 second or more after flowing the at least one molecule into the at least one microchannel or microchamber.

19. The method of claim 17, wherein the selected point in time comprises about 0.1 second to about 10 seconds or more after flowing the at least one molecule into the at least one microchannel or microchamber.

20. The method of claim 17, wherein the selected point in time comprises about 0.1 second to about 1.0 minute or more after flowing the at least one molecule into the at least one microchannel or microchamber.

21. The method of claim 1, wherein heating comprises elevating the temperature of the at least one molecule to a selected temperature.

22. The method of claim 21, wherein the selected temperature comprises about 10 °C to about 60 °C or more.

23. The method of claim 21, wherein the selected temperature comprises about 10 °C to about 90 °C or more.

24. The method of claim 21, wherein the selected temperature comprises about 10 °C to about 100 °C or more.

25. The method of claim 1, wherein heating comprises elevating the temperature of the at least one molecule in the at least one microchannel or microchamber by one or more of: joule heating or non-joule heating.

26. The method of claim 25, wherein joule heating comprises flowing a selectable electric current through the at least one microchannel or microchamber, thereby elevating the temperature.

5 27. The method of claim 26, wherein the joule heating occurs over the entire length of the at least one microchannel or microchamber.

28. The method of claim 26, wherein the joule heating occurs over a selected zone of the at least one microchannel or microchamber.

10 29. The method of claim 28, wherein the joule heating comprises flowing a selectable electric current through at least a first section of the at least one microchannel or microchamber and through at least a second section of the at least one microchannel or microchamber; wherein the first section of the microchannel comprises a first cross-section and the second section of the microchannel comprises a second cross-section and the first cross-section is greater in size than the second cross-section, thereby causing the second cross-section to have a higher electrical resistance than the first cross-section, thereby further causing the second cross-section to have a higher temperature than the first cross-section.

15 30. The method of claim 26, wherein joule heating further comprises controlling at least one of: the selectable current or the electrical resistance; thereby controlling the temperature.

20 31. The method of claim 26, wherein the selectable current comprises direct current or alternating current or a combination of direct current and alternating current.

25 32. The method of claim 25, wherein non-joule heating comprises elevating the temperature of the at least one microchannel or microchamber through use of an internal or an external heat source.

33. The internal or external heat source in claim 32, wherein the heat source comprises a thermal heating block.

34. The method of claim 32, wherein the non-joule heating occurs over the entire length of the at least one microchannel or microchamber or where non-joule heating occurs over a selected zone of the at least one microchannel or microchamber.

35. The method of claim 1, wherein the detectable property comprises
5 fluorescence or emitted light.

36. The method of claim 1, wherein the detectable property comprises a change in the total free energy of a system comprising the at least one molecule in the at least one microchannel or microchamber.

37. The method of claim 1, wherein the detectable property comprises a
10 change in a dielectric property of the at least one molecule in the at least one microchannel or microchamber.

38. The method of claim 1, wherein the detectable property comprises a change in an applied electric current needed to maintain a constant temperature of the at least one molecule in the at least one microchannel or microchamber.

39. The method of claim 35, wherein detecting comprises that the at least
15 one molecule comprises a first molecule and a second molecule, and wherein the first molecule comprises a fluorescence indicator dye or a fluorescence indicator molecule.

40. The method of claim 39, wherein the fluorescence indicator dye or
20 fluorescence indicator molecule binds or associates with the second molecule by binding to hydrophobic or hydrophilic residues on the second molecule.

41. The method of claim 40, wherein detecting further comprises
exciting the fluorescence indicator dye or fluorescence indicator molecule, thereby
creating an excited fluorescence indicator dye or excited fluorescence indicator molecule.

42. The method of claim 41, wherein detecting further comprises
25 discerning an emission or quenching event of the excited fluorescence indicator dye or fluorescence indicator molecule and wherein detecting further comprises measuring a

fluorescence of the excited fluorescence indicator dye or excited fluorescence indicator molecule.

43. The method of claim 35, wherein detecting comprises that the at least one molecule comprises one or more protein or one or more polypeptide.

5 **44.** The method of claim 43, wherein detecting further comprises exciting one or more tryptophan residues in the at least one molecule, thereby creating one or more excited tryptophan residue.

10 **45.** The method of claim 44, wherein detecting further comprises discerning an emission or quenching event of the one or more excited tryptophan residue and wherein detecting further comprises measuring a fluorescence of the one or more excited tryptophan residue.

15 **46.** The method of claim 35, wherein detecting comprises use of one or more of the group comprising: fluorescence polarization, fluorescence resonance energy transfer, or fluorescence lifetime imaging microscopy.

20 **47.** The method of claim 36, wherein detecting comprises discerning the change in the total free energy of the system and wherein detecting further comprises measuring the change in the total free energy of the system.

25 **48.** The method of claim 36, wherein detecting comprises discerning the change in the total free energy of the system and wherein detecting further comprises measuring the amount of electric current needed to maintain a selected temperature or temperatures.

49. The method of claim 1, wherein generating the thermal property curve comprises that the at least one molecule comprises a first molecule and at least a second molecule, and wherein the first molecule comprises a fluorescence indicator dye or fluorescence indicator molecule.

50. The method of claim 49, wherein the at least second molecule comprises two or more of: an enzyme, a ligand, a peptide nucleic acid, a cofactor, a

receptor, a substrate, a protein, a polypeptide, a nucleic acid, a single-stranded nucleic acid, a double-stranded nucleic acid, an antibody, an antigen, or an enzyme complex.

51. The method of claim 50, wherein generating the thermal property curve comprises measuring a fluorescence of the first molecule in the presence of the at least second molecule as a function of temperature.

52. The method of claim 51, wherein generating the thermal property curve further comprises measuring the melting temperature (T_m) of the at least second molecule.

53. The method of claim 51, wherein a change in the fluorescence of the first molecule is proportional to a change in the physical property of the at least second molecule due to a change in temperature.

54. The method of claim 49, wherein generating the thermal property curve further comprises generating a control curve by measuring a fluorescence of the first molecule in the presence of the second molecule as a function of temperature, and wherein the second molecule comprises one of: a protein, a polypeptide, an enzyme, an enzyme complex, a nucleic acid, a single-stranded nucleic acid, a double-stranded nucleic acid, a ligand, a peptide nucleic acid, a cofactor, a receptor, an antibody, an antigen, or a substrate.

55. The method of claim 1, wherein generating the thermal property curve comprises that the at least one molecule comprises a first molecule and at least a second molecule and wherein the first molecule comprises one or more of: a protein, a polypeptide, or an enzyme

56. The method of claim 55, wherein the first molecule further comprises one or more tryptophan residues.

57. The method of claim 56, wherein the at least second molecule comprises one or more of: an enzyme, a ligand, a peptide nucleic acid, a cofactor, a

receptor, a substrate, a protein, a polypeptide, a nucleic acid, a single-stranded nucleic acid, a double-stranded nucleic acid, an antibody, an antigen, or an enzyme complex.

58. The method of claim 57, wherein generating the thermal property curve comprises measuring a fluorescence of the one or more tryptophan residues present
5 in the first molecule while in the presence of the at least second molecule as a function of temperature.

59. The method of claim 58, wherein generating the thermal property curve comprises measuring the melting temperature point of the at least one molecule.

60. The method of claim 58, wherein a change in fluorescence of the
10 tryptophan residues is proportional to a change in the physical property of the at least first molecule due to change in temperature.

61. The method of claim 60, wherein generating the thermal property curve further comprises generating a control curve by measuring a fluorescence of the first molecule as a function of temperature without the presence of the at least second
15 molecule.

62. The method of claim 1, wherein generating the thermal property curve comprises that the at least one molecule comprises a first molecule and at least a second molecule.

63. The method of claim 62, wherein the first molecule and at least
20 second molecule are chosen from the group comprising: a protein, a polypeptide, an enzyme, an enzyme complex, a nucleic acid, a single-stranded nucleic acid, a double-stranded nucleic acid, a ligand, a peptide nucleic acid, a cofactor, a receptor, an antibody, an antigen, or a substrate.

64. The method of claim 63, wherein generating the thermal property
25 curve comprises measuring a change in the total free energy of the system comprising the first molecule and the at least second molecule in the at least one microchannel or

microchamber as a function of temperature when the first molecule is in the presence of the at least second molecule.

5 **65.** The method of claim 64, wherein generating the thermal property curve further comprises generating a control curve by measuring the change in the total free energy of the system as a function of temperature without the presence of the at least second molecule.

10 **66.** The method of claim 63, wherein generating the thermal property curve comprises measuring a change in a dielectric property of the first molecule as a function of temperature when the first molecule is in the presence of the at least second molecule.

15 **67.** The method of claim 66, wherein generating the thermal property curve further comprises generating a control curve by measuring the change in the dielectric property of the first molecule as a function of temperature without the presence of the at least second molecule.

20 **68.** The method of claim 63, wherein generating the thermal property curve comprises measuring a change in an applied electric current needed to maintain a constant temperature of the first molecule as a function of temperature when the first molecule is in the presence of the at least second molecule.

25 **69.** The method of claim 68, wherein generating the thermal property curve further comprises generating a control curve by measuring the change in the applied electric current needed to maintain a constant temperature of the first molecule as a function of temperature without the presence of the at least second molecule.

70. The method of claim 1, further comprising determining a peak temperature in the microfluidic device through construction of a thermal property curve for one or more molecules of known T_m .

71. The method of claim 70, wherein the one or more molecule comprises a first molecule and at least a second molecule, which first molecule and at least second molecule bind to each other over a known temperature range.

72. The method of claim 71, wherein the first molecule and the at least
5 second molecule are chosen from the group consisting of: biotin, biotin-4-fluorescein, fluorescein biotin, avidin, streptavidin, and neutravidin.

73. The method of claim 71, wherein the first molecule and the at least second molecule together comprise a complementary double-stranded nucleic acid molecule of known sequence and known T_m .

10 74. The method of claim 73, wherein the first molecule and the at least second molecule are each labeled with an indicator molecule.

75. The method of claim 73, wherein the first molecule and the at least second molecule are each labeled with a different indicator molecule, thus allowing detection of a separation of the first molecule and the at least second molecule.

15 76. An integrated system or microfluidic device comprising:

- (a) a body structure having at least one fluidic microchannel or microchamber disposed therein;
- (b) a fluid direction system for controllably moving one or more reagent into and through the at least one fluidic microchannel or microchamber;
- 20 (c) an energy source for controllably heating at least one of the one or more reagents in the at least one microchannel or microchamber;
- (d) a source of at least one fluorescence indicator dye or fluorescence indicator molecule, wherein the source is fluidly coupled to the at least one microchannel or microchamber;
- 25 (e) a source of at least one molecule, wherein the source is fluidly coupled to the at least one microchannel or microchamber;
- (f) an excitation source for exciting the at least one fluorescence indicator dye or at least one fluorescence indicator molecule, resulting in at least one excited fluorescence

indicator dye or at least one excited fluorescence indicator molecule;
(g) a detector proximal to the body structure for detecting a change in at least one physical property of the at least one molecule; and,
(h) a computer operably coupled to the detector, wherein the computer comprises an instruction set for acquiring data from the detector and for constructing thermal melt curves and/or control curves from the data.

77. The integrated system or microfluidic device in claim 76, wherein during operation, the fluid direction system controllably determines a selection of one or more reagent to be added to the at least one microchannel or microchamber.

78. The integrated system or microfluidic device in claim 76, wherein during operation, the fluid direction system controllably determines an amount of one or more reagent to be added to the at least one microchannel or microchamber.

79. The integrated system or microfluidic device in claim 76, wherein during operation, the fluid direction system controllably determines a time at which one or more reagent is to be added to the at least one microchannel or microchamber.

80. The integrated system or microfluidic device in claim 76, wherein during operation, the fluid direction system controllably determines a speed at which one or more reagent is to be added to the at least one microchannel or microchamber.

81. The integrated system or microfluidic device of claim 76, wherein the energy source, during operation, elevates the temperature of the at least molecule in the at least one microchannel or microchamber by one or more of: joule heating or non-joule heating.

82. The integrated system or microfluidic device of claim 81, wherein joule heating comprises flowing a selectable electric current through the at least one microchannel or microchamber; thereby elevating the temperature.

83. The integrated system or microfluidic device of claim 81, wherein the joule heating occurs over the entire length of the at least one microchannel or microchamber.

84. The integrated system or microfluidic device of claim 81, wherein
5 the joule heating occurs over a selected zone of the at least one microchannel or microchamber.

85. The integrated system or microfluidic device of claim 84, wherein the joule heating comprises flowing a selectable electric current through at least a first section of the at least one microchannel or microchamber and through at least a second
10 section of the at least one microchannel or microchamber; wherein the first section of the microchannel comprises a first cross-section and the second section of the microchannel comprises a second cross-section and the first cross-section is greater in size than the second cross-section, thereby causing the second cross-section to have a higher electrical resistance than the first cross-section, thereby further causing the second cross-section to
15 have a higher temperature than the first cross-section.

86. The integrated system or microfluidic device of claim 81, wherein joule heating further comprises applying a selectable current through the at least one microchannel or microchamber and controlling at least one of the selectable current or the electrical resistance; thereby controlling the temperature.

87. The integrated system or microfluidic device of claim 86, wherein
20 the selectable current comprises direct current or alternating current or a combination of direct current and alternating current.

88. The integrated system or microfluidic device of claim 81, wherein non-joule heating comprises elevating the temperature of the at least one microchannel or
25 microchamber through an internal or external heat source.

89. The internal or external heat source in claim 88, wherein the heat source comprises a thermal heating block.

90. The integrated system or microfluidic device of claim 81, wherein the non-joule heating occurs over the entire length of the at least one microchannel or microchamber or where source heating occurs over a selected zone of the at least one microchannel or microchamber.

5 91. The integrated system or microfluidic device in claim 76, wherein the at least one fluorescence indicator dye or fluorescence indicator molecule binds to one or more hydrophobic amino acid residue, one or more hydrophilic amino acid residue, or a combination thereof.

10 92. The integrated system or microfluidic device of claim 91, wherein the fluorescence indicator dye or fluorescence indicator molecule comprises 1-anilino-naphthalene-8-sulfonate.

15 93. The integrated system or microfluidic device in claim 76, wherein the at least one fluorescence indicator dye or fluorescence indicator molecule intercalates into one or more nucleic acid polymers.

20 94. The integrated system or microfluidic device of claim 76, wherein the at least one fluorescence indicator molecule comprises at least one tryptophan residue.

25 95. The integrated system or microfluidic device of claim 76, wherein the source of the at least one molecule comprises one or more of: a high-through-put format, a low-through-put format, or a multiplex format.

96. The integrated system or microfluidic device of claim 76, wherein the excitation source for exciting the at least one fluorescence indicator dye or fluorescence indicator molecule comprises a light source.

97. The integrated system or microfluidic device in claim 96, wherein the excitation source for exciting the at least one fluorescence indicator dye or

fluorescence indicator molecule comprises one of more of: a tungsten-halogen lamp, a xenon-arc lamp, a mercury lamp, a laser, or a fiber optic cable.

5 **98.** The integrated system or microfluidic device in claim 76, wherein the detector comprises one or more of: a fiber optic probe, a charge coupled device, a fluorescence imaging camera, a photomultiplier, a photodiode, a calorimeter, or a fluorescence polarization sensor.

10 **99.** The integrated system or microfluidic device in claim 76, wherein the detector comprises the ability to detect fluorescence or emitted light from the excited at least one fluorescence indicator dye or from the excited at least one fluorescence indicator molecule.

15 **100.** The integrated system or microfluidic device in claim 76, wherein the detector comprises the ability to detect a change in a total free energy of a system comprising the at least one molecule in the at least one microchannel or microchamber.

20 **101.** The integrated system or microfluidic device in claim 76, wherein the detector comprises the ability to detect a change in a dielectric property of the at least one molecule in the at least one microchannel or microchamber.

25 **102.** The integrated system or microfluidic device in claim 76, wherein the detector comprises the ability to detect a change in an applied electric current needed to maintain a constant temperature of the at least one molecule in the at least one microchannel or microchamber.

103. The integrated system or microfluidic device in claim 76, wherein the computer determines a peak temperature achieved in the at least one microfluidic channel through construction of a thermal property curve for one or more molecules of known T_m .

104. The integrated system or microfluidic device in claim 103, wherein the one or more molecule comprises a first molecule and at least a second molecule,

which first molecule and at least second molecule bind to each other over a known temperature range.

105. The integrated system or microfluidic device in claim 104, wherein the first molecule and the at least second molecule are chosen from the group consisting of: biotin, biotin-4-fluorescein, fluorescein biotin, avidin, streptavidin, and neutravidin.

106. The integrated system or microfluidic device in claim 104, wherein the first molecule and the at least second molecule together comprise a complementary double-stranded nucleic acid molecule of known sequence and known T_m .

107. The integrated system or microfluidic device in claim 106, wherein the first molecule and the at least second molecule are each labeled with an indicator molecule.

108. The integrated system or microfluidic device in claim 106, wherein the first molecule and the at least second molecule are each labeled with a different indicator molecule, thus allowing detection of a separation of the first molecule and the at least second molecule.